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(71) Applicant *(for all designated States except US)*: BIOGEN, INC. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : WALLNER, Barbara, P. [US/US]; 7 Centre Street, Cambridge, MA 02139 (US). BENJAMIN, Christopher, D. [US/US]; 2 Oak Hill Lane, Beverly, MA 01915 (US).

(74) Agents: McDONNELL, John, J. et al.; Allegretti & Witcoff, Ltd., Ten South Wacker Drive, Chicago, IL 60606 (US).

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(57) Abstract

Methods of improving tolerance of transplanted xenograft tissue or allograft tissue in mammals, including humans, by the administration of LFA-3 or CD2 binding proteins.

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METHODS OF IMPROVING ALLOGRAFT OR XENOGRAFT TOLERANCE BY ADMINISTRATION OF AN LFA-3 OR CD2 BINDING PROTEIN

This application is a continuation-in-part of application Serial No. 07/772,705, filed October 7, 1991, now pending.

TECHNICAL FIELD OF INVENTION

The present invention relates to methods of improving tolerance of transplanted xenograft tissue or allograft tissue by administration of LFA-3 or CD2 binding proteins in mammals, including humans.

BACKGROUND OF THE INVENTION

An allograft is tissue that is transplanted between genetically nonidentical members of the same species. Allografts of organs such as the heart, kidney, liver, pancreas, cornea, bone marrow, lung and skin have become an increasingly successful and accepted medical practice for the treatment of various end stage diseases. The resulting increase in demand for transplants, unfortunately, has not been matched by an increase in the present donor supply, and efforts to increase the supply of human donors are not predicted to match the rising demand for human organs. For example, only 2,000 of the 14,000 patients per year who are eligible for a cardiac allograft actually receive a heart transplant in the United States (Rose, "Risks Of

Cardiac Transplantation", Ann. Thorac. Surg., 47, p. 615 (1989)).

Consequently, interest has increased in alternative sources for donor organs. One such alternative source is xenografts, which are transplants of tissue from one species to another species.

A problem for both allografts and xenografts is rejection of the donor graft tissue by the recipient. Graft rejection is the result of a complicated and not fully understood chain of events in the immune system. There are generally two facets of the immune response: 1) a cell mediated response, primarily comprising cytotoxic T cells which attack and kill foreign cells or virus-infected cells; and 2) a humoral response, comprising the activation of B cells to plasma cells which secrete antibodies specific for foreign macromolecules.

Graft rejection is histologically characterized by the progressive infiltration of mononuclear cells, including lymphocytes, into the foreign tissue. The increased presence of these cells precedes the destruction of the graft by several days. Sensitized T lymphocytes, therefore, appear to be the principal initiators of the rejection process.

T lymphocytes play a major role in the immune response by interacting with target and antigen-presenting cells. For example, T lymphocyte-mediated killing of target cells is a multi-step process involving, initially, adhesion of cytolytic T lymphocytes (the effector cells) to target cells, such as graft endothelium. Also, helper T lymphocytes help initiate the immune response by adhesion to antigen-presenting cells within the graft tissue.

These interactions of T lymphocytes with target and antigen-presenting cells are highly specific

and depend on the recognition of an antigen on the surface of a target or antigen-presenting cell by one of the many specific antigen receptors on the surface of T lymphocytes.

The receptor-antigen interaction of T lymphocytes and other cells is also facilitated by various T lymphocyte surface proteins, e.g., the antigen-receptor complex CD3 and accessory molecules such as CD4, LFA-1, CD8, and CD2. It is also affected by accessory molecules such as LFA-3, ICAM-1 and MHC that are expressed on the surface of the target or antigen-presenting cells.

The interaction between CD2 and LFA-3 remains poorly understood with respect to activation of T cell activity. Recent studies have suggested that there is a specific interaction between CD2 (a T lymphocyte accessory adhesion molecule) and LFA-3 (a target cell and antigen presenting cell accessory molecule) which mediates T lymphocyte adhesion to the target or antigen presenting cell. This cell-cell adhesion has been implicated in the initiation of T lymphocyte functional responses (Dustin et al., "Purified Lymphocyte Function Associated Antigen 3 Binds To CD2 And Mediates T lymphocyte Adhesion," J. Exp. Med., 165, pp. 677-92 (1987); Springer et al., "The Lymphocyte Function-associated LFA-1, CD2, and LFA-3 Molecules: Cell Adhesion Receptors of the Immune System", Ann. Rev. Immunol., 5, pp. 223-52 (1987)). The LFA-3/CD2 interaction also plays a role in mediating T lymphocyte interactions with thymic epithelial cells, in antigen-independent and dependent conjugate formation and in T lymphocyte rosetting with erythrocytes (see, e.g., Seed et al., "Molecular Cloning Of The CD2 Antigen, the T-Cell Erythrocyte Receptor, By a Rapid Immunoselection

Procedure", Proc. Natl. Acad. Sci. USA, 84, pp. 3365-69 (1987)).

LFA-3, which is found on the surface of a wide variety of cells, including human erythrocytes, has become the subject of a considerable amount of study to further elucidate its role in various T lymphocyte interactions (see, e.g., Krensky et al., "The Functional Significance, Distribution, and Structure of LFA-1, LFA-2, and LFA-3: Cell Surface Antigen Associated with CTL-Target Interactions", J. Immunol., 131(2), pp. 611-16 (1983); Shaw et al., "Two Antigen-Independent Adhesion Pathways Used by Human Cytotoxic T-cell Clones", Nature, 323, pp. 262-64 (1986)). Two natural forms of LFA-3 have been identified. One form of LFA-3 ("transmembrane LFA-3") is anchored in the cell membrane by a transmembrane hydrophobic domain. cDNA encoding this form of LFA-3 has been cloned and sequenced (see, e.g., Wallner et al., "Primary Structure of Lymphocyte Function-Associated Antigen-3 (LFA-3)", J. Exp. Med., 166, pp. 923-32 (1987)). Another form of LFA-3 is anchored to the cell membrane via a covalent linkage to phosphatidylinositol ("PI")-containing glycolipid. This latter form has been designated "PI-linked LFA-3", and cDNA encoding this form of LFA-3 has also been cloned and sequenced (Wallner et al., PCT publn. WO 90/02181).

The human CD2 (T11) molecule is a 50 kD surface glycoprotein expressed on >95% of thymocytes and virtually all peripheral T lymphocytes. Biochemical analyses using specific monoclonal antibodies have suggested that CD2 is T lineage-specific and exists on the cell surface in several differentially glycosylated forms (Howard et al., "A Human T Lymphocyte Differentiation Marker Defined by

Monoclonal Antibodies that Block E-Rosette Formation", J. Immunol., 126, pp. 2117-22 (1981); Brown et al., in Leukocyte Typing III, ed. McMichael, Oxford University Press, pp. 110-12 (1987); Sayre et al., "Molecular Cloning and Expression of T11 cDNAs Reveals a Receptor-Like Structure on Human T lymphocytes", Proc. Natl. Acad. Sci. USA, 84, pp. 2941-45 (1987)). The sequence of a human CD2 gene has been reported (Seed and Aruffo, "Molecular Cloning of the CD2 Antigen, the T-cell Erythrocyte Receptor, by a Rapid Immunoselection Procedure", Proc. Natl. Acad. Sci. USA, 84, pp. 3365-69 (1987); Sayre et al., supra (1987). Soluble CD2 polypeptides having an LFA-3 binding domain have been reported (PCT publ. WO 90/08187).

Monoclonal antibodies to CD2, for example TS2/18, $T11_1$, $T11_2$, $T11_3$, and to LFA-3, for example TS2/9, have also been reported (see, e.g., Hughes et al., "The Endothelial Cell as a Regulator of T-Cell Function", Immunol. Reviews, 117, pp. 85-102 (1990); Meuer, "An Alternative Pathway of T-Cell Activation: A Functional Role for the 50 kd T11 Sheep Erythrocyte Receptor Protein", Cell, 36, pp. 897-906 (1984); Sanchez-Madrid et al., "Three Distinct Antigens Associated with Human T-Lymphocyte-Mediated Cytolysis: LFA-1, LFA-2, and LFA-3", Proc. Natl. Acad. Sci. USA, 79, pp. 7489-93 (1982)).

Suppression of the immune response to prevent graft rejection has previously been effected by drugs, such as prednisone, cyclosporine, azathioprine or cyclophosphamide, which nonspecifically block cell-mediated responses. Irradiation has also been used to destroy T and B lymphocytes that could react against the transplanted graft tissue. Immunosuppression with the above techniques, however, cannot produce antigen-specific tolerance and, therefore, greatly increases

the patient's susceptibility to opportunistic infection. In addition, other detrimental side effects will occur with chronic use of the above immunosuppression techniques, For example, chronic cyclosporine treatment is associated with a high incidence of renal toxicity, hypertension and malignant neoplasm.

Cytotoxic T lymphocyte mediated responses are controlled by cyclosporine or prednisone, but immune suppressive therapy is ineffectual for humoral rejection episodes. Currently, there is no therapeutic intervention for humoral rejection.

To date, therefore, conventional methods and therapeutic agents have not proved to be satisfactory for improving tolerance of xenografts or allografts. Accordingly, the need still exists for a process which avoids the disadvantages of the conventional methods and agents while providing an effective method for decreasing the severity of rejection of graft tissue.

SUMMARY OF THE INVENTION

The present invention generally solves many of the problems referred to above. It, for the first time, provides a method for improving tolerance of transplanted allograft tissue or xenograft tissue in a mammal. The method of this invention comprises the steps of administering to a mammal, preferably a human, a graft tissue and an LFA-3 or CD2 binding protein. The methods of the invention will preferably be used to improve tolerance of cardiac and renal xenografts and allografts. The methods of this invention are superior to previously available therapies for improving graft tolerance for many reasons, including avoidance of undesirable side effects such as increased

susceptibility to opportunistic infection, renal toxicity, hypertension and malignant neoplasm.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1 and 2 illustrate T cell dependent B cell activation assay results for two baboons injected with an anti-LFA-3 monoclonal antibody (1E6) and one baboon injected with a non-specific isotype matched control monoclonal antibody (MOPC21). Immunoglobulin production as measured by OD units in an ELISA assay is reflected on the y axes. The number of days after the initial injection of anti-LFA-3 monoclonal antibody is illustrated on the x axes.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, an "LFA-3 binding protein" is a protein comprising one or more polypeptides capable of binding to LFA-3. LFA-3 binding proteins include immunoglobulin light chains, immunoglobulin heavy chains and antigen-binding fragments thereof. The component polypeptides of an LFA-3 binding protein composed of more than one polypeptide may optionally be disulfide-bound or otherwise covalently crosslinked. Accordingly, LFA-3 binding proteins include intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof), wherein the light chains of the immunoglobulin may be of types kappa or lambda. Such binding proteins also include portions of intact immunoglobulins that retain LFA-3-binding specificity, for example, Fab fragments, Fab' fragments, $F(ab')_2$ fragments, F(v) fragments, heavy chain monomers or dimers, light chain monomers or dimers, dimers consisting of one heavy and one light chain, and the like.

Also contemplated within the term "LFA-3 binding protein" are soluble CD2 polypeptides and derivatives thereof, including fusions, that bind to LFA-3. As used herein, a "soluble CD2 polypeptide" is a CD2 polypeptide incapable of anchoring itself in a cell membrane. Such soluble polypeptides include, for example, CD2 polypeptides that lack a sufficient portion of their membrane-spanning domain to anchor the polypeptide or are modified such that the membrane-spanning domain is nonfunctional. Soluble CD2 polypeptides bind to a naturally occurring LFA-3 polypeptide and are encoded by (a) a naturally occurring mammalian CD2 DNA sequence (e.g., SEQ ID NO:5), (b) a DNA sequence degenerate to a naturally occurring CD2 DNA sequence or (c) a DNA sequence that hybridizes to one of the foregoing DNA sequences under conditions equivalent to about 20°C to 27°C below T_m and 1 M sodium chloride. Such soluble CD2 polypeptides are well known. For example, several are described in PCT WO 90/08187, which is herein incorporated by reference.

As used herein, a "CD2 binding protein" is a protein comprising one or more polypeptides capable of binding to CD2. CD2 binding proteins include immunoglobulin light chains, immunoglobulin heavy chains and antigen-binding fragments thereof. The component polypeptides of a CD2 binding protein composed of more than one polypeptide may optionally be disulfide-bound or otherwise covalently crosslinked. Accordingly, CD2 binding proteins include intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof), wherein the light chains of the immunoglobulin may be of types kappa or lambda. Such binding proteins also include portions of intact immunoglobulins that retain CD2-binding specificity,

for example, Fab fragments, Fab' fragments, F(ab')2 fragments, F(v) fragments, heavy chain monomers or dimers, light chain monomers or dimers, dimers consisting of one heavy and one light chain, and the like.

Also contemplated within the term "CD2 binding protein" are soluble LFA-3 polypeptides or derivatives thereof, including fusions, that bind to CD2. As defined herein, CD2 binding proteins include fusions of soluble LFA-3 polypeptides and immunoglobulin regions, such as LFA3TIP (described infra). As used herein, a "soluble LFA-3 polypeptide" is a LFA-3 polypeptide incapable of anchoring itself in a cell membrane. Such soluble polypeptides include, for example, LFA-3 polypeptides that lack a sufficient portion of their membrane-spanning domain to anchor the polypeptide or are modified such that the membrane-spanning domain is nonfunctional. Soluble LFA-3 polypeptides bind to a naturally occurring CD2 polypeptide and are encoded by (a) a naturally occurring mammalian LFA-3 DNA sequence (e.g. SEQ ID NO:1 or SEQ ID NO:3, (b) a DNA sequence degenerate to a naturally occurring LFA-3 DNA sequence or (c) a DNA sequence that hybridizes to one of the foregoing DNA sequences under conditions equivalent to about 20°C to 27°C below T_m and 1 M sodium chloride. Such soluble LFA-3 polypeptides are well known. For example, several are described in United States patent 4,956,281, which is herein incorporated by reference.

As used herein, a "humanized recombinant antibody" is an antibody, produced by recombinant DNA technology, in which some or all of the amino acids of a human immunoglobulin light or heavy chain not required for antigen binding have been substituted for

the corresponding amino acids from a nonhuman mammalian immunoglobulin light or heavy chain.

As used herein, a "chimeric recombinant antibody" is an antibody produced by recombinant DNA technology, in which all or part of the hinge and constant regions of an immunoglobulin light chain, heavy chain or both, have been substituted for the corresponding regions from another immunoglobulin light chain or heavy chain.

As used herein, "improving tolerance" of transplanted graft tissue is decreasing the severity of or eliminating one or more of the general characteristics of graft rejection. Such characteristics evidence immune response directed against the graft (foreign) tissue and include, for example, progressive infiltration of mononuclear cells, such as lymphocytes, into the foreign tissue, production of lymphocytotoxic antibodies, cytolysis, necrosis, vasculitis, hemorrhage and fibrosis. Another readily observable indication of improved tolerance will be prolonged survival of transplanted graft tissue in a recipient as compared to a non-immunosuppressed recipient (control).

Graft Tissue

The methods of this invention are useful in improving tolerance in mammals, including humans, of transplanted allograft tissue or xenograft tissue. They comprise the steps of administering to the mammal a graft tissue and an LFA-3 or CD2 binding protein. Such grafts include allografts and xenografts of tissues derived from sources including the heart, kidney, liver, pancreas, cornea, bone marrow, lung, skin and blood. Such tissues include portions of the organs mentioned above and subfractions of blood.

Preferably, the methods of this invention are used for cardiac allografts and xenografts, and renal allografts and xenografts. The methods of the invention can be practiced on any mammal, preferably humans.

In selecting graft tissue, a variety of factors should be considered. These include, for example, a minimization of genetic disparity to the extent possible, ABO blood group compatibility, HLA compatibility, the availability of donor tissue, the immune status of the patient and size of the donor organ. Specifically, in the case of cardiac and renal allografts or xenografts, the donor organ should be anatomically compatible and physiologically competent to support the organ function requirements of the recipient. Surgical protocols used for various graft transplants are well known.

While not wishing to be bound by theory, applicants believe that the LFA-3 and CD2 binding proteins used in the methods of this invention are prophylactic and therapeutic for inducing tolerance of the xenografts or allografts because they inhibit T cell activation. This inhibition typically occurs when the LFA-3 or CD2 binding protein inhibits the LFA-3/CD2 interaction. However, certain LFA-3 and CD2 binding proteins used in this invention may inhibit T cell activation without inhibiting the LFA-3/CD2 interaction.

Preferred LFA-3 and CD2 binding proteins for use in the methods of this invention are effective to inhibit T cell activation.

The utility in the methods of this invention of specific LFA-3 or CD2 binding proteins may easily be determined by assaying their ability to inhibit the LFA-3/CD2 interaction, their ability to inhibit T cell activation or both.

The ability to inhibit the LFA-3/CD2 interaction may be assayed, for example, using a simple cell binding assay that permits visual (under magnification) evaluation of the ability of the putative inhibitor to inhibit the interaction between LFA-3 and CD2 on cells expressing these molecules. Jurkat cells are preferred as the $CD2^+$ substrate and sheep red blood cells or human JY cells are preferred as the $LFA\text{-}3^+$ substrate. The binding characteristics of binding proteins useful in this invention may be assayed in several known ways, such as by radiolabeling the binding protein (e.g., with ^{35}S or ^{125}I) and then contacting the labeled binding protein with $CD2^+$ or $LFA\text{-}3^+$ cells, as appropriate. Binding characteristics may also be assayed using an appropriate enzymatically labelled secondary antibody. Rosetting competition assays, such as those described in Seed et al., Proc. Natl. Acad. Sci. USA, 84, pp. 3365-69 (1987) may also be used.

The ability of LFA-3 and CD2 binding proteins to inhibit T cell activation may be determined in any number of conventional T cell activation assays. These include, for example, assays which assess the ability of the binding protein to inhibit T cell proliferation or cytokine secretion in response to mitogens or activating monoclonal antibodies directed to other cell surface proteins (see, e.g., Moingeon et al., "The Structural Biology of CD2", Immunological Rev., 111, pp. 111-44 (1989)).

LFA-3 and CD2 Binding Proteins

Many types of LFA-3 and CD2 binding proteins are useful in the methods of this invention, including monoclonal antibodies, recombinant antibodies, chimeric recombinant antibodies, humanized recombinant

antibodies, soluble LFA-3 and CD2 polypeptides and LFA-3 and CD2 mimetic agents, as well as derivatized (e.g., fused to another polypeptide) or truncated forms of any of the foregoing.

A. Antibodies

The LFA-3 and CD2 binding proteins useful in this invention include monoclonal antibodies, recombinant antibodies, chimeric recombinant antibodies, humanized recombinant antibodies, and antigen binding portions thereof. Preferably, the antibodies are monoclonal antibodies.

It is more preferable to use a monoclonal anti-LFA-3 antibody produced by a hybridoma selected from the group of hybridomas having accession numbers ATCC HB 10693 (1E6), ATCC HB 10694 (HC-1B11), ATCC HB 10695 (7A6), and ATCC HB 10696 (8B8), or the monoclonal antibody known as TS2/9 (Sanchez-Madrid et al., "Three Distinct Antigens Associated With Human T-Lymphocyte-Mediated Cytolysis: LFA-1, LFA-2 and LFA-3", Proc. Natl. Acad. Sci. USA., 79, pp. 7489-93 (1982)). Most preferably, the monoclonal anti-LFA-3 antibody is produced by the hybridoma having accession number ATCC HB 10693 (1E6).

Among the anti-CD2 antibodies, preferable monoclonal antibodies include monoclonal antibodies known as the $T11_1$ epitope antibodies, including TS2/18 (Sanchez-Madrid et al., supra, (1982)).

The technology for producing monoclonal antibodies is well known. Briefly, an immortal cell line (typically myeloma cells) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a preparation comprising a given antigen, and the culture supernatants of the resulting hybridoma cells are screened for antibodies against the antigen. See

generally, Kohler et al., "Continuous Cultures Of Fused Cells Secreting Antibody Of Predefined Specificity", Nature, 256, pp. 495-97 (1975). Useful immunogens for the purpose of this invention include LFA-3-expressing or CD2-expressing cells, as well as cell free preparations containing LFA-3, CD2, or counter receptor-binding fragments thereof (i.e., CD2 fragments that bind to LFA-3 or LFA-3 fragments that bind to CD2). Also useful are derivatized forms of LFA-3, CD2 or portions thereof, such as fusion proteins consisting of a soluble LFA-3 polypeptide fused to at least portions of immunoglobulin hinge and constant domains (e.g., LFA3TIP, described infra).

Immunization may be accomplished using standard procedures. The unit dose and immunization regimen depend on the species of mammal immunized, its immune status, the body weight of the mammal, etc. Typically, the immunized mammals are bled and the serum from each blood sample is assayed for particular antibodies using appropriate screening assays. For example, useful anti-LFA-3 and anti-CD2 antibodies may be identified by testing the ability of the immune serum to block sheep red blood cell rosetting of Jurkat cells, which results from the presence of LFA-3 and CD2 on the respective surfaces of these cells, screening for the ability to inhibit T cell activation in vitro or screening for both. The lymphocytes used in the production of hybridoma cells typically are isolated from immunized mammals whose sera have already tested positive for the presence of the desired antibodies using such screening assays.

Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive

to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium").

Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol (PEG 3350). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridomas producing a desired antibody are detected by screening the hybridoma culture supernatants, for example, for the ability to bind to LFA-3 or CD2, or for their ability to block Jurkat cell adhesion to sheep red blood cells. Useful hybridomas may also be identified by screening for the ability to inhibit T cell activation. Subcloning of the hybridoma cultures by limiting dilution is typically performed to ensure monoclonality.

To produce anti-LFA-3 and anti-CD2 monoclonal antibodies, hybridoma cells that tested positive in such screening assays are cultured in a nutrient medium under conditions and for a time sufficient to allow the hybridoma cells to secrete the monoclonal antibodies into the culture medium. Tissue culture techniques and culture media suitable for hybridoma cell culture are well known. The conditioned hybridoma culture supernatant may be collected and the desired antibodies optionally further purified by well known methods.

Alternatively, the desired antibody may be produced by injecting the hybridoma cells into the peritoneal cavity of a Pristane-primed [2,6,10,14-tetramethylpentadecane (Aldridge Chemical Co., Milwaukee, Wisconsin)] mouse. The hybridoma cells proliferate in the peritoneal cavity and secrete the antibody which accumulates in ascites fluid. The

antibody may be harvested by withdrawing the ascites fluid from the peritoneal cavity with a syringe.

LFA-3 and CD2 binding proteins useful in the present invention may also be recombinant antibodies produced by host cells transformed with DNA encoding immunoglobulin light and heavy chains of a desired antibody, or LFA-3 or CD2-binding portions thereof. Recombinant antibodies may be produced by well known genetic engineering techniques. See, e.g., United States patent 4,816,397, which is incorporated herein by reference.

For example, recombinant antibodies may be produced by cloning cDNA or genomic DNA encoding the immunoglobulin light and heavy chains of the desired antibody from a hybridoma cell that produces an antibody useful in this invention. The cDNA or genomic DNA encoding those polypeptides is then inserted into expression vectors so that both DNA sequences are operatively linked to one or more transcriptional and translational expression control sequences. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. Typically, both DNA sequences are inserted into the same expression vector, although the two DNA sequences may also be inserted into different expression vectors.

Prokaryotic or eukaryotic host cells may be used as expression hosts. Expression in eukaryotic host cells is preferred because such cells are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. However, any antibody produced that is inactive due to improper folding may be renaturable according to well known methods (Kim and Baldwin, "Specific Intermediates in the Folding Reactions of Small Proteins and the

Mechanism of Protein Folding", Ann. Rev. Biochem., 51, pp. 459-89 (1982)). It is possible that the host cells will produce portions of intact antibodies useful in this invention, such as light chain dimers or heavy chain dimers.

It will be understood that variations on the above procedure are useful in the present invention. For example, it may alternatively be desired to transform a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an anti-LFA-3 or anti-CD2 antibody. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for LFA-3 or CD2 counter receptor binding. The molecules expressed from such truncated DNA molecules are useful in the methods of this invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are specific for LFA-3 or CD2 and the other heavy and light chain are specific for an antigen other than LFA-3 or CD2, or for another epitope of LFA-3 or CD2.

Chimeric recombinant antibodies may be produced by transforming a host cell with a suitable expression vector comprising DNA encoding the desired immunoglobulin light and heavy chains in which all or some of the DNA encoding the hinge and constant regions of the heavy and/or the light chain have been substituted with DNA from the corresponding region of an immunoglobulin light or heavy chain of a different species. When the original recombinant antibody is nonhuman and the anti-LFA-3 or anti-CD2 antibody will be administered to a human, substitution of corresponding human sequences is preferred. An exemplary chimeric recombinant antibody has mouse variable regions and human hinge and constant regions.

See generally, United States patent 4,816,397 and Morrison et al., "Chimeric Human Antibody Molecules: Mouse Antigen-Binding Domains With Human Constant Region Domains", Proc. Natl. Acad. Sci. USA, 81, pp. 6851-55 (1984).

Humanized recombinant anti-LFA-3 or anti-CD2 antibodies may be produced by transforming a host cell with a suitable expression vector comprising DNA encoding the desired nonhuman immunoglobulin light and heavy chains in which all or some of the DNA encoding amino acids not involved in antigen binding have been substituted with DNA from the corresponding region of a desired human immunoglobulin light or heavy chain. See generally, Jones et al., "Replacing The Complementarity-Determining Regions In A Human Antibody With Those From A Mouse", Nature, 321, pp. 522-25 (1986) and European patent publication 0 239 400.

Anti-LFA-3 and anti-CD2 antibodies that are not intact are also useful in this invention, and may be derived from any of the antibodies described above. For example, antigen-binding fragments, as well as full-length monomeric, dimeric or trimeric polypeptides derived from the above-described antibodies are themselves useful. Useful binding proteins of this type include Fab fragments, Fab′ fragments, $F(ab')_2$ fragments, F(v) fragments, heavy chain monomers or dimers, light chain monomers or dimers, dimers consisting of one heavy and one light chain, and the like.

Antibody fragments may also be produced by chemical methods, e.g., by cleaving an intact antibody with a protease, such as pepsin or papain, and optionally treating the cleaved product with a reducing agent. Alternatively, useful fragments may be produced by using host cells transformed with truncated heavy

and/or light chain genes. Heavy and light chain monomers may be produced by treating an intact antibody with a reducing agent, such as dithiothreitol, followed by purification to separate the chains. Heavy and light chain monomers may also be produced by host cells transformed with DNA encoding either the desired heavy chain or light chain, but not both. See, e.g., Ward et al., "Binding Activities Of A Repertoire Of Single Immunoglobulin Variable Domains Secreted From Escherichia coli", Nature, 341, pp. 544-46 (1989); Sastry et al., "Cloning Of The Immunological Repertoire in Escherichia coli For Generation Of Monoclonal Catalytic Antibodies: Construction Of A Heavy Chain Variable Region-Specific cDNA Library", Proc. Natl. Acad. Sci. USA, 86, pp. 5728-32 (1989).

B. Soluble CD2 and LFA-3 Polypeptides

The LFA-3 and CD2 binding proteins useful in the methods of the present invention include soluble CD2 and LFA-3 polypeptides. Soluble LFA-3 polypeptides are preferred.

Soluble LFA-3 polypeptides may be derived from the transmembrane form of LFA-3, particularly the extracellular domain (e.g., AA_1-AA_{187} of SEQ ID NO:2). Such polypeptides are described in United States patent 4,956,281 and co-pending, commonly assigned United States patent applications 07/667,971 and 07/770,967, which are herein incorporated by reference. Preferred soluble LFA-3 polypeptides include polypeptides consisting of AA_1-AA_{92} of SEQ ID NO:2, AA_1-AA_{80} of SEQ ID NO:2, AA_{50}-AA_{65} of SEQ ID NO:2 and AA_{20}-AA_{80} of SEQ ID NO:2. A bacteriophage comprising a DNA sequence encoding SEQ ID NO:2 (i.e., SEQ ID NO:1) is deposited with American Type Culture Collection, Rockville, Maryland, under the accession number ATCC 75107.

Soluble LFA-3 polypeptides may also be derived from the PI-linked form of LFA-3, such as those described in PCT patent application WO 90/02181. A vector comprising a DNA sequence encoding PI-linked LFA-3 (i.e., SEQ ID NO:3) is deposited with American Type Culture Collection, Rockville, Maryland, under the accession number ATCC 68788. Since the PI-linked form of LFA-3 and the transmembrane form of LFA-3 have identical amino acid sequences through the entire extracellular domain, the preferred soluble LFA-3 polypeptides derived from PI-linked LFA-3 are the same as those derived from the transmembrane form of LFA-3.

Soluble CD2 polypeptides may be derived from full length CD2, particularly the extracellular domain (e.g., AA_1-AA_{185} of SEQ ID NO:6). Such polypeptides may comprise all or part of the extracellular domain of CD2. Suitable soluble CD2 polypeptides are described in PCT WO 90/08187, which is herein incorporated by reference.

The production of the soluble polypeptides useful in this invention may be achieved by a variety of methods known in the art. For example, the polypeptides may be derived from intact transmembrane LFA-3 or CD2 molecules or an intact PI-linked LFA-3 molecule by proteolysis using specific endopeptidases in combination with exopeptidases, Edman degradation, or both. The intact LFA-3 molecule or the intact CD2 molecule, in turn, may be purified from its natural source using conventional methods. Alternatively, the intact LFA-3 or CD2 may be produced by known recombinant DNA techniques using cDNAs (see, e.g., U.S. Patent 4,956,281 to Wallner et al.; Aruffo and Seed, Proc. Natl. Acad. Sci. USA, 84, pp. 2941-45 (1987); Sayre et al., Proc. Natl. Acad. Sci. USA, 84, pp. 2941-45 (1987)).

Preferably, the soluble polypeptides useful in the present invention are produced directly, thus eliminating the need for obtaining an entire LFA-3 molecule or an entire CD2 molecule as a starting material. This may be achieved by conventional chemical synthesis techniques or by well-known recombinant DNA techniques wherein only those DNA sequences which encode the desired polypeptides are expressed in transformed hosts. For example, a DNA sequence which encodes the desired soluble LFA-3 polypeptide or soluble CD2 polypeptide may be synthesized by chemical means using an oligonucleotide synthesizer. Such oligonucleotides are designed based on the amino acid sequence of the desired soluble LFA-3 polypeptide or soluble CD2 polypeptide. Specific DNA sequences coding for the desired polypeptide also can be derived from the full length DNA sequence by isolation of specific restriction endonuclease fragments or by PCR synthesis of the desired region.

The soluble LFA-3 and CD2 polypeptides may be isolated from the fermentation or culture of transfected host cells and purified using any of a variety of conventional methods. One of skill in the art may select the most appropriate isolation and purification techniques.

While recombinant DNA techniques are the preferred method of producing useful soluble CD2 polypeptides or soluble LFA-3 polypeptides having a sequence of more than 20 amino acids, shorter CD2 or LFA-3 polypeptides having less than about 20 amino acids are preferably produced by conventional chemical synthesis techniques. Synthetically produced polypeptides useful in this invention can advantageously be produced in extremely high yields and can be easily purified.

C. LFA-3 And CD2 Mimetic Agents

Among the LFA-3 and CD2 binding proteins useful in the methods of this invention are LFA-3 and CD2 mimetic agents. These agents are peptides, semi-peptidic compounds or non-peptidic compounds which bind to CD2 (LFA-3 mimetic) or to LFA-3 (CD2 mimetic) and inhibit the CD2/LFA-3 interaction, inhibit T cell activation or both.

Such mimetic agents may be produced by synthesizing a plurality of peptides (e.g., 5-20 amino acids in length), semi-peptidic compounds or non-peptidic, organic compounds, and then screening those compounds for their ability to inhibit the CD2/LFA-3 interaction or for their ability to inhibit T cell activation or both. See generally United States patent 4,833,092; Scott and Smith, "Searching for Peptide Ligands with an Epitope Library", Science, 249, pp. 386-90 (1990); and Devlin et al., "Random Peptide Libraries: A Source of Specific Protein Binding Molecules", Science, 249, pp. 404-07 (1990), which are herein incorporated by reference.

D. Derivatized LFA-3 And CD2 Binding Proteins

Also useful in the methods of this invention are derivatized forms, including fusions or hybrids, of the foregoing LFA-3 and CD2 binding proteins in which, for example, any of the LFA-3 or CD2 binding proteins described herein are functionally linked (by chemical coupling, genetic fusion or otherwise) to one or more of the same or different LFA-3 and CD2 binding proteins, to pharmaceutical agents, or to both.

One type of derivatized binding protein is produced by crosslinking two or more LFA-3 or CD2 binding proteins (of the same type or of different

types). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Illinois.

Another possibility for cross-linking takes advantage of the PI linkage signal sequence in PI-linked LFA-3, or fragments thereof. Specifically, DNA encoding the PI-linkage signal sequence (e.g., AA_{162}-AA_{212} of SEQ ID NO:4) is ligated downstream of DNA encoding a desired polypeptide, preferably a soluble LFA-3 polypeptide. If this construct is expressed in an appropriate eukaryotic cell, the cell will recognize the PI linkage signal sequence and will covalently link PI to the polypeptide. The hydrophobic property of the PI may then be exploited to form micellar aggregates of the polypeptides.

Also useful are LFA-3 and CD2 binding proteins linked to one or more pharmaceutical agents (e.g., a fusion or hybrid protein). Useful pharmaceutical agents include biologically active peptides, polypeptides and proteins, such as antibodies specific for a polypeptide other than LFA-3 or CD2. Other useful pharmaceutical agents include immunosuppressants, for example, cyclosporine A, prednisone, FK506, methotrexate, steroids, and retinoids.

Preferred derivatized binding proteins include recombinantly produced polypeptides in which a soluble LFA-3 polypeptide, soluble CD2 polypeptide, or a peptidyl CD2 or peptidyl LFA-3 mimetic agent is fused to all or part of an immunoglobulin heavy chain hinge region and all or part of an immunoglobulin heavy chain

constant region. Such fusion proteins are expected to exhibit prolonged serum half-lives and to facilitate binding protein dimerization.

Preferred polypeptides for preparing such fusion proteins are soluble LFA-3 polypeptides, most preferably a soluble LFA-3 polypeptide selected from the group consisting of AA_1-AA_{92} of SEQ ID NO:2, AA_1-AA_{80} of SEQ ID NO:2, AA_{50}-AA_{65} of SEQ ID NO:2 and AA_{20}-AA_{80} of SEQ ID NO:2.

A bacteriophage comprising a DNA sequence encoding SEQ ID NO:2 (i.e., SEQ ID NO:1) is deposited with the American Type Culture Collection, Rockville, Maryland, under the accession number ATCC 75107.

The most preferred fusion proteins of this type contain the amino terminal 92 amino acids of mature LFA-3, the C-terminal 10 amino acids of a human IgG_1 hinge region containing the two cysteine residues thought to participate in interchain disulfide bonding, and the C_H2 and C_H3 regions of a human IgG_1 heavy chain constant domain (e.g., SEQ ID NO:8). This fusion protein is referred to herein as "LFA3TIP." A plasmid, pSAB152, encoding an exemplary LFA3TIP is deposited with American Type Culture Collection, Rockville, Maryland, under the accession number ATCC 68720. The DNA sequence of the pSAB152 insert is SEQ ID NO:7.

One way of producing LFA3TIP for use in the methods of this invention is described in co-pending, commonly assigned United States patent application 07/770,967. Generally, conditioned culture medium of COS7 cells transfected with pSAB152 was concentrated using an AMICON S1Y30 spiral cartridge system (AMICON, Danvers, Massachusetts) and subjected to Protein A-Sepharose 4B (Sigma, St. Louis, Missouri) chromatography. The bound proteins were eluted and

subjected to Superose-12 (Pharmacia/LKB, Piscataway, New Jersey) gel filtration chromatography.

Superose-12 fractions containing LFA3TIP with the least amount of contaminating proteins, as determined on SDS-PAGE gels and by Western blot analysis, (see, e.g., Towbin et al., Proc. Natl. Acad. Sci. USA, 74, pp. 4350-54 (1979); Antibodies: A Laboratory Manual, pp. 474-510 (Cold Spring Harbor Laboratory (1988)), were pooled and concentrated in a YM30 Centricon (AMICON). LFA3TIP was detected on Western blots using a rabbit anti-LFA-3 polyclonal antiserum, followed by detectably labeled goat anti-rabbit IgG. The purified LFA3TIP of COS7 cells was a dimer of two monomeric LFA-3-Ig fusion proteins, connected by disulfide bonds.

Pharmaceutical Compositions And Methods According To This Invention

The methods according to this invention improve tolerance of transplanted allograft tissue or xenograft tissue by administering to a mammal the graft tissue and one or more LFA-3 or CD2 binding proteins, including derivatized forms thereof. The LFA-3 or CD2 binding proteins may alternatively be administered as part of a pharmaceutical composition.

Useful pharmaceutical compositions will comprise one or more LFA-3 or CD2 binding proteins, including derivatized forms thereof, typically in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable carrier" is meant a carrier that does not cause an allergic reaction or other untoward effect in patients to whom it is administered.

Suitable pharmaceutically acceptable carriers include, for example, one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol

and the like, as well as combinations thereof. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the LFA-3 or CD2 binding protein.

The LFA-3 or CD2 binding proteins or compositions useful in this invention will preferably be administered in an "effective amount," meaning an amount capable of improving tolerance to an allograft or xenograft as defined herein.

It will be apparent to those of skill in the art that the effective amount of LFA-3 or CD2 binding protein will depend, inter alia, upon the administration schedule, the unit dose administered, whether the LFA-3 or CD2 binding protein is administered in combination with other therapeutic agents, the immune status and health of the patient, the therapeutic or prophylactic activity of the particular LFA-3 or CD2 binding protein administered and its serum half-life.

The pharmaceutical compositions may further be used in conjunction with general immunosuppressive agents. These include, for example, cyclosporine, azathioprine and steroids, such as Depo-Medrol (methylprednisolone acetate), Solumederol (methylprednisolone sodium succinate), and prednisone, administered in amounts effective to suppress immune response in the mammal being treated. For example, cyclosporine may be administered at 2-25 mg/kg/day p.o. starting the day before surgery, azathioprine may be administered at 50-200 mg/day, Solumederol may be administered at 125 mg i.v. at the time of transplantation and on the first post-operative day, prednisone may be administered at 1 mg/kg/day p.o.

starting on the second post-operative day or Depo-Medrol may be administered at 0.8 mg/kg/day i.m. starting on the second post-operative day. The above dosages will, of course, be varied by the practitioner depending upon factors well known to those of skill in the art. In general, when used in conjunction with an LFA-3 or CD2 binding protein, it will be desired to use the lowest possible effective concentration of such immunosuppressive agents.

The pharmaceutical compositions may further comprise other therapeutic or prophylactic agents. The LFA-3 or CD2 binding protein and the other active agent may be in the form of a single conjugated molecule. Conjugation of the two components may be achieved by standard cross-linking techniques well known in the art. A single molecule may also take the form of a recombinant fusion protein.

The additional immunosuppressive, therapeutic or prophylactic agents may be administered in single dosage form with the LFA-3 or CD2 binding protein, in a multiple dosage form separately from the LFA-3 or CD2 binding protein, but contemporaneously, or in a multiple dosage form wherein the components are administered separately but sequentially. Such combination therapies may advantageously utilize lower dosages of the immunosuppressive, therapeutic or prophylactic agents.

The pharmaceutical compositions or LFA-3 or CD2 binding proteins may be in a variety of forms. These include, for example, solid, semi-solid and liquid dosage forms, such as tablets, pills, powders, liquid solutions, dispersions or suspensions, liposomes, suppositories, injectable and infusible solutions. The preferred form depends on the intended mode of administration and therapeutic application.

The preferred form is injectable or infusible solutions.

Typically, the LFA-3 or CD2 binding protein will be suspended in a sterile saline solution for therapeutic uses. The pharmaceutical compositions may alternatively be formulated to control release of the active ingredients or to prolong their presence in a recipient's system. Numerous suitable drug delivery systems are known and include, e.g., hydrogels, hydroxymethylcellulose, microcapsules, liposomes, microemulsions, microspheres, and the like.

In accordance with this invention, a mammal that is to receive transplanted graft tissue and an LFA-3 binding protein is administered a dose between about 0.01 and about 10 mg LFA-3 binding protein per kg body weight, more preferably between about 0.1 and about 5 mg LFA-3 binding protein per kg body weight, and most preferably between about 0.1 and about 2 mg LFA-3 binding protein per kg body weight.

A mammal that is to receive transplanted graft tissue and a CD2 binding protein is administered a dose between about 0.01 and about 10 mg CD2 binding protein per kg body weight, more preferably between about 0.01 and about 2 mg CD2 binding protein per kg body weight, and most preferably between about 0.01 and about 1 mg CD2 binding protein per kg body weight.

The LFA-3 or CD2 binding protein or composition should be administered about once per day until, within the judgment of the practitioner, the danger of rejection of the allograft or xenograft tissue has diminished. The length of administration of the LFA-3 or CD2 binding protein or composition is dependent upon the mammal's acceptance of the graft tissue. General clinical indications of rejection will vary with the particular organ transplanted. However,

fever, malaise and organ dysfunction are typical clinical indications of rejection. Symptoms of organ dysfunction depend upon the organ transplanted, but are characterized by well known and recognized indicia to those of skill in the art.

The success of the treatment may be measured by a variety of methods including biopsies, such as incisional myocardial biopsy or percutaneous endomyocardial biopsy to determine the extent of lymphocyte infiltration, blood assays to determine the extent of lymphocytotoxic antibody production or a mixed lymphocyte reaction (see, e.g., Krensky et al., J. Immunol., 131, pp. 611-16 (1983); Bradley, "Mixed Lymphocyte Responses", in Selected Methods in Cellular Immunology (Mishell and Shiigi, eds.), pp. 162-64 (W.H. Freeman and Co., San Francisco 1980)). In the case of renal transplants, biopsies can be taken to determine the extent of mononuclear cell infiltration and proliferation, or necrosis of the arterial endothelium and media in the graft tissue. (Cosimi et al., J. Immunol., 144, pp. 4604-12 (1990)).

The method of the present invention, in a preferred embodiment for allograft tissue, comprises administering the LFA-3 or CD2 binding protein once per day for two consecutive days before the transplant and once per day for one to ten consecutive days after the transplant. More preferably, the LFA-3 or CD2 binding protein is administered once per day for two consecutive days before the transplant and once per day for two consecutive days after the transplant.

The method of the present invention, in a preferred embodiment for xenograft tissue, comprises administering, before the transplant, an LFA-3 or CD2 binding protein contemporaneously with tissue from the xenograft source. As used herein, "contemporaneously"

when referring to the administration of tissue from a xenograft source (other than the graft tissue) and an LFA-3 or CD2 binding protein, will mean that their administration occurs near enough in time to allow the binding protein to bind to the tissue from the xenograft source at an effective level to inhibit a significant immune response. Preferably, the binding protein is bound to the tissue from the xenograft source at saturating levels. In a preferred embodiment of this invention, administration of one occurs within approximately zero to six hours of the other. Most preferably, the tissue from the xenograft source and the LFA-3 or CD2 binding protein are administered within approximately zero to one hour of each other. Either may be administered first. It is preferable, however, that the binding protein be administered prior to tissue from the xenograft source.

In an alternate embodiment of the present invention, the contemporaneous administration is followed by the administration of LFA-3 or CD2 binding protein before the transplant.

More preferably, the LFA-3 or CD2 binding protein is administered before the xenograft transplant once per day for two consecutive days, then contemporaneously with tissue from the xenograft source once per day for one day, and then once per day for one to ten consecutive days. If the xenograft source species and recipient species are unusually discordant, it may be necessary to administer the LFA-3 or CD2 binding protein contemporaneously with tissue from the xenograft source once per day for two consecutive days according to the above schedules. In a preferred embodiment, the binding protein is administered once per day for five to ten consecutive days after the contemporaneous administration and before the

transplant according to the above schedules. Most preferably, the contemporaneous administration of the LFA-3 or CD2 binding protein and tissue from the xenograft source is simultaneous.

Although not wishing to be bound by theory, applicants administer tissue from the xenograft source to the mammal contemporaneously with LFA-3 or CD2 binding protein with the intent of inhibiting the development of a population of activated cells specifically reactive against that tissue. The contemporaneous administration of LFA-3 or CD2 binding proteins induces tolerance to the specific subset of antigens carried by cells from the specific xenograft source. Accordingly, it will be understood that any tissue from the xenograft source may be appropriate, however blood cells from the xenograft source are preferred. Such tissue should be administered in an amount sufficient to elicit an immune response. The preferred method of administration of tissue from the xenograft source is intravenous. The administration of between about 1×10^6 to about 1×10^8 whole blood cells most preferably will serve as the tissue from the xenograft source. It will be recognized, however, that lower or higher dosages and other administration schedules may be employed.

The LFA-3 or CD2 binding protein or pharmaceutical composition may be administered intravenously, intramuscularly, subcutaneously, intra-articularly, intrathecally, periostally, orally, topically or by inhalation. Ordinarily, intravenous (i.v.) or intramuscular (i.m.) administration will be preferred, however, more localized administrations in the area of transplantation may be more desirable in some cases due to the wide range of cells in the body that express LFA-3.

In a preferred embodiment of the method of the present invention, the graft tissue is perfused with an effective amount of LFA-3 or CD2 binding protein before implantation into the mammal. Most preferably, the graft tissue is perfused with enough LFA-3 or CD2 binding protein to saturate all CD2 or LFA-3 sites on the graft tissue before implantation into the mammal.

In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only, and are not to be construed as limiting the scope of the invention in any manner.

EXAMPLES

Example 1

Purification Of Anti-LFA-3 Monoclonal Antibody 1E6 and Monoclonal Antibody MOPC21

1E6 hybridoma cells (ATCC HB 10693) were grown in RPMI 1640 medium supplemented with 2% fetal calf serum, 150 μg/ml streptomycin and 50 μg/ml gentamicin (GIBCO Life Technologies, Gaithersburg, Maryland) in three 40 liter stirred glass vessels (Bellco, # 196536000) at 37°C for 7 - 10 days. The conditioned media was pooled and collected into 100 liter carboys (NALGENE). Sodium azide was added to make the pooled suspension 0.02% final concentration. The cell debris was removed through a 5 μ filter cartridge (Polygard, #CN5001E06, Millipore, Bedford, Massachusetts) followed by a 0.3 μ filter cartridge (Polygard, #CN0301E06, Millipore, Bedford, Massachusetts) at room temperature. The clarified supernatant was concentrated 50 to 100 fold using a YM30 S10 spiral filter cartridge (AMICON, Danvers, Massachusetts) at 4°C. The concentrate from 50 liters

of conditioned media was diluted with two volumes of equilibration buffer (3 M glycine, 1.5 M sodium chloride, pH 8.9) and passed through 90 ml of Protein A-Sepharose (Schleicher and Schuell, Keene, New Hampshire) overnight by gravity at 4°C.

The column was washed with equilibration buffer and the bound proteins were subsequently eluted with 100 mM sodium citrate, pH 3.0. The eluted fractions were collected into 1/10 fraction volume of 1 M HEPES, pH 7.8. A280 readings of the fractions were taken and the fractions containing the eluted protein were pooled and stored at -70°C. Protein A-purified 1E6 was prepared from a total of about 200 liters of conditioned media. The various pools were thawed, combined and concentrated to about 10 mg/ml protein in a 2 liter Amicon stirred cell using a YM30 filter (AMICON, Danvers, Massachusetts). The concentrated material was divided into five 100 ml aliquots. Each aliquot was passed through a 1 liter Superose-6 gel filtration column (Pharmacia, Piscataway, New Jersey) developed in phosphate buffered saline at room temperature. The peak fractions containing 1E6 were pooled and stored at -70°C. When all the material was processed, the pools were thawed, combined and adjusted to 2-3 mg/ml protein with phosphate buffered saline. The final material was divided into 15 ml aliquots and stored at -70°C until use.

MOPC21 was purified from ascites purchased from the Sigma Chemical Corporation (St. Louis, Missouri) by diluting the ascites into the "Protein A loading buffer" of 3 M glycine, 1.5 M sodium chloride, pH 8.9, and passing it over 25 ml of Protein A-Sepharose (Schleicher and Schuell, Keene, New Hampshire) at room temperature. The column was washed with the loading buffer until the optical density at

280 nm returned to a baseline level. The bound IgG was eluted with 50 mM sodium acetate, pH 3.0, at room temperature and dialyzed overnight against 50 volumes of phosphate buffered saline at 4°C. After dialysis, the MOPC21 was passed through a 1 liter Superose-6 gel filtration column (Pharmacia, Piscataway, New Jersey) developed in phosphate buffered saline at room temperature. The peak fractions containing MOPC21, were pooled, adjusted with phosphate buffered saline to a final concentration of 2 mg/ml protein and stored in 30 mg aliquots at -70°C until use. All preparations contained less than 10 units/ml endotoxin as determined using the commercially available kit Chromogenic LAL (Whittaker M.A. Bioproducts, Walkersville, Maryland). Except as otherwise noted, all purification steps were performed at room temperature.

Example 2

Effect Of Administration Of Anti-LFA-3 Monoclonal Antibody 1E6 On Lymphocyte Function

A. Administration And Sampling Protocols

Two outbred, adult baboons A and B (Papio anubis) were given bolus injections of 1.45 mg/kg of the purified anti-LFA-3 monoclonal antibody 1E6, i.v., by portacatheter once daily for five consecutive days. Baboon A weighed 12 kg. Baboon B weighed 9.5 kg. As a control, another adult baboon C, 9.4 kg, was injected with equal amounts of the non-specific, isotype-matched mouse monoclonal antibody MOPC21 (Sigma Chemical Corp., St. Louis, Missouri). Blood was drawn from the baboons once or twice before the first injection of antibody and then, daily for five days, four hours after each injection. Blood was also drawn on day 8, day 11 and day 14, where day 1 is the day of the first injection. This administration and sampling protocol was used for

all of the assays described in this example, unless otherwise stated.

B. Toxicology Study with Anti-LFA-3 Monoclonal Antibody 1E6

The general toxicity of anti-LFA-3 monoclonal antibody 1E6 and the potential effect on the physical condition, hematology and blood chemistry of baboons was evaluated. The general physical condition of the baboons remained unchanged throughout the study. No obvious or immediate side effects could be observed. Hematology and blood chemistries generally remained normal. In particular, Na^+, Cl^-, K^+, creatine, blood urea nitrogen and liver enzymes AST and ALT levels all remained with normal limits. In addition, blood cell counts, including hematocrit, white blood cells, lymphocytes, monocytes, segmented neutrophils and eosinophils, generally stayed within normal ranges. However, baboon B showed a substantial decrease in segmented neutrophils after day five.

C. Serum Levels of Anti-LFA-3 Monoclonal Antibody 1E6 and Control MOPC21

Serum was prepared from blood drawn four hours after antibody injection. For the baboons injected with 1E6 (baboons A and B), additional serum was collected at the 24 hour time point, just before the antibody injections on days one to five. Serum was also collected on days 8, 11 and 14. Serum levels of MOPC21 and 1E6 were determined by measurement of mouse IgG levels with an ELISA using microtiter plates coated with goat anti-mouse IgG (Jackson Immunoresearch, Malvern, Pennsylvania). These ELISAs were standardized using MOPC21 and 1E6 purified as described in Example 1. Serum levels of 1E6 capable of binding to LFA-3 (i.e., "active" 1E6) were measured with an ELISA

using microtiter plates coated with a soluble LFA-3 polypeptide consisting of AA_1-AA_{184} of LFA-3 (see U.S. patent 4,956,281, which is herein incorporated by reference). This ELISA was also standardized with 1E6 purified as described in Example 1. In all of the above ELISA assays, binding of 1E6 or MOPC21 to microtiter plates was detected using a second goat anti-mouse antibody that was labelled with alkaline phosphatase (Jackson Immunoresearch, Malvern, Pennsylvania). The bound immunoglobulin was quantified by the colorimetric conversion of the alkaline phosphatase substrate pNPP to its colored product using a Thermomax (Molecular Devices, Palo Alto, California). The ELISA reader was at a wavelength of 405 nm. (Data not shown.)

Serum levels of 1E6 and MOPC21 peaked between day four and day five (about 40-80 µg/ml antibody) and returned to pre-injection levels between day eight and day eleven. Serum levels of 1E6, 24 hours after injection, consistently decreased between 50% and 80% of the level at four hours after injection for serum collected on days 1-5. In comparison, MOPC21 levels decreased only between 10% and 20% after 24 hours. The percentage of active 1E6 in serum varied between 40% and 70%. 1E6 serum levels were higher in baboon B as compared to baboon A (9.5 kg compared to 12 kg body weight), possibly as a result of different tissue space distribution.

The titer of anti-1E6 antibodies in the treated baboon serum was determined by ELISA. Purified 1E6 was coupled to microtiter plates and serum from each bleed was assayed at increasing dilutions. (Data not shown.)

In both 1E6 injected baboons A and B, anti-1E6 antibodies were detected after the injection

as early as day eleven. Anti-MOPC21 titers were detected using anti-mouse IgG coated assay plates and showed the same kinetics as anti-1E6. (Data not shown.)

D. T cell Activation Assays In Vitro

To determine the effect of 1E6 injections on T cell activation in vitro, peripheral blood lymphocytes were isolated from antibody-injected baboons and assayed for T cell dependent B cell activation and for T cell proliferation in response to phytohemagglutinin or activating anti-CD2 monoclonal antibodies. For each of these assays, peripheral blood lymphocytes were isolated on Ficoll-Hypaque (Pharmacia, Piscataway, New Jersey), according to the manufacturer's suggested protocol. Peripheral blood lymphocytes were stored overnight in tissue culture medium containing 10% fetal calf serum at room temperature prior to each assay.

1. T cell Dependent B-Cell Activation Assay

The T cell dependent B cell activation to immunoglobulin secretion can be blocked by anti-LFA-3 antibodies (MOPC21 is used as a control).

Peripheral blood mononuclear cells were purified from whole blood on Ficoll Hypaque density medium (Pharmacia, Piscataway, New Jersey), according to the manufacturer's instructions. Adherent macrophages were removed by incubating the mononuclear cells on plastic dishes for 45 minutes at 37°C. The nonadherent lymphocytes were washed in a physiologically compatible culture medium (RPMI 1640, GIBCO Life Technologies, Gaithersburg, Maryland), determined to contain minimal macrophages by FACS analysis on a FACStar (Becton Dickinson Corporation,

Mountainview, California) using fluorescently labelled antibodies specific for macrophage/monocyte cell surface antigens and cultured in 96-well round bottom plates (RPMI 1640 supplemented with 10% fetal calf serum, 2 mM glutamine, 5 x 10-5 M ß-mercaptoethanol and nonessential amino acids (GIBCO Life Technologies, Gaithersburg, Maryland)).

In this culture, T cells activate B cells to secrete immunoglobulin. The B cells are not activated in the absence of T cells. The immunoglobulin secreted into the culture medium was measured by sampling culture medium on day seven and day twelve after the initiation of the culture. The supernatant (cell free) samples were analyzed for baboon immunoglobulin using an ELISA in which the assay plates were coated with goat anti-human immunoglobulin (Jackson Immunoresearch, Malvern, Pennsylvania), which also recognizes baboon immunoglobulin, but does not bind to immunoglobulin present in the fetal calf serum or to mouse immunoglobulins. The immunoglobulins from the culture supernatants that were bound to the goat anti-human immunoglobulin-coated plates were detected using a second goat anti-human immunoglobulin reagent to which an enzyme, alkaline phosphatase, had been coupled (Jackson Immunoresearch, Malvern, Pennsylvania). The bound immunoglobulin was quantified by the colorimetric conversion of the alkaline phosphatase substrate pNPP (para-nitrophenylphosphate) to its colored product. Substrate conversion was measured in a Thermomax (Molecular Devices, Palo Alto, California) ELISA reader at a wavelength of 405 nm.

The results of these experiments are shown in Figures 1 and 2. Figure 1 displays relative absorbance units at 405 nm from the ELISA assay for assays performed on baboon B (1E6) lymphocytes from days 0,

1-5, 8, 11 and 14. Figure 2 displays relative absorbance units at 405 nm from the ELISA assay for assays performed on lymphocytes from baboons A (1E6) and C (MOPC21) on days 0, 1-5, 8 and 11.

For baboon B, T cell dependent B cell Ig production decreased on the second day of 1E6 injections and remained at about 35% of the day zero value through day eleven (Figure 1).

For baboon A, Ig production was higher on days 1-11 as compared to the level before the injection. This is likely due to the lower 1E6 serum level achieved in baboon A versus baboon B. If Ig production levels observed on days one through four are taken as a base value, then a 40% inhibition of Ig secretion was observed on day five, and a 20% inhibition on day eleven (Figure 2).

In baboon C, after injection with MOPC21, peripheral blood lymphocytes showed increased levels of Ig production between days two and eleven as compared to the level on day zero.

2. <u>T cell Proliferation Assay</u>

In a T cell proliferation assay, we measured the ability of activating anti-CD2 monoclonal antibodies or phytohemagglutinin ("PHA") to cause proliferation of T cells isolated from baboons A, B and C on days 0, 1-5, 8, 11 and 14. 1×10^5 peripheral blood lymphocytes per well were incubated (1) with anti-CD2 monoclonal antibodies $T11_1$ and $T11_3$ at a 1:900 dilution of ascites fluid, (2) in medium alone, or (3) with PHA (Sigma Chemical Corporation, St. Louis, Missouri) (10 μg/ml) for three days. After three days, cells were labelled with 1 μCi/well ^{3}HdT for 18 hours and then harvested. (Data not shown.)

Peripheral blood lymphocytes from baboon B showed no increase of ^{3}HdT incorporation in response to activating anti-CD2 monoclonal antibodies and very low proliferative activity in medium on days zero to fourteen.

Peripheral blood lymphocytes from baboon A responded to anti-CD2 monoclonal antibodies and PHA. After day four, proliferation in response to those agents was inhibited about nine fold and remained low until at least day fourteen.

Peripheral blood lymphocytes from baboon C, the MOPC21 control, showed very low proliferative activity at all time points tested, under all conditions.

The significance of the data obtained is not clear because of irreproducibility of T cell proliferation in baboon C and day zero results for baboons A, B and C.

Example 3

Effect Of Administration Of LFA3TIP On Lymphocyte Function

A. Administration And Sampling Protocols

Two outbred, adult baboons (4.6 and 7.4 kg) (*Papio anubis*) were given bolus injections of 3 mg/kg of purified LFA3TIP (obtained from Biogen, Inc., Cambridge, Massachusetts), i.v., by portacatheter once daily for five consecutive days. Blood was drawn from the baboons once before the first injection of antibody and then, daily for five days, 24 hours after each injection. Blood was also drawn on day 8, day 10, day 15, and day 22, where day 1 is the day of the first injection. This administration and sampling protocol was used for all of the assays described in this example, unless otherwise stated.

B. Toxicology Study With LFA3TIP

The general toxicity of LFA3TIP and its potential effect on the physical condition, hematology and blood chemistry of baboons were evaluated. The general physical condition of the baboons remained unchanged throughout the study. No obvious or immediate side effects could be observed. Hematology and blood chemistries generally remained normal. In particular, Na^+, Cl^-, K^+, creatine, blood urea nitrogen and liver enzymes AST and ALT levels all remained within normal limits. In addition, blood cell counts, including hematocrit, white blood cells, lymphocytes, monocytes, segmented neutrophils, and eosinophils, generally stayed within normal ranges. The ratio of CD4/CD8 expressing cells also stayed within normal ranges.

Plasma levels of LFA3TIP 10 days after the last injection were still about 32% of the LFA3TIP levels immediately following the last injection, which indicates a much longer half-life than generally observed with murine monoclonal antibodies. Fluorescent labeling of CD4 and CD8 expressing cells indicated that about 10% of $CD4^+$ cells and about 90% of $CD8^+$ cells were still coated with LFA3TIP 10 days after the last injection.

Example 4

Baboon Cardiac Allograft Model

A. 1E6 Treatment

An experimental primate cardiac allograft model where baboon hearts were transplanted heterotopically in a nonfunctioning position into the necks of ABO-matched outbred baboons (Papio anubis) was used to assess the effect of anti-LFA-3 monoclonal

antibody 1E6 on allograft rejection. The protocol used was substantially as described in Michler et al., "Techniques For Primate Heterotopic Cardiac Xenotransplantation," J. Med. Primatol., 14, pp. 357-62 (1985), except that an allograft not a xenograft was performed.

Purified 1E6 prepared as described above was injected into one adult baboon (weight 32 kg) at a dose of 5 mg/kg, starting on day one, for 2 consecutive days before the transplant. On the third day, a cardiac heterotopic allograft transplant was performed with a heart from a young, 3 kg baboon. One dose of 5 mg/kg of 1E6 was injected on the day of the transplant and then once a day for ten consecutive days. Blood samples were collected two days before transplantation, prior to injection. Blood samples were also collected coincident with transplantation and on the fifth, tenth, sixteenth, nineteenth and twenty-first day after transplantation. An assay for total 1E6 serum levels and the proportion of active 1E6 in the serum, i.e., the percentage of 1E6 capable of binding to LFA-3, was performed as described in Example 2C. No general immunosuppressive agents were administered to the baboon.

The graft was palpated on a daily basis and monitored by palpation and visual assessment of heart beat. Electrocardiograms were performed on a weekly basis. A percutaneous endomyocardial biopsy was performed on the sixteenth day after transplantation. All blood chemistry and cell counts performed on the above described blood samples were within the normal limits.

Untreated control cardiac allografts in this model system were rejected a mean of 9±3 days (n=5) after implantation in non-immunosuppressed baboons

(Rose et al., "Cardiac Xenotransplantation", Progress In Cardiovascular Diseases, 33, pp. 105-17 (1990)). Rejection is defined, for the purposes of this model system, as swelling and hardening of the heart, and cessation of heart beat as measured by an electrocardiogram. In addition, progressive infiltration of lymphocytes in the myocardium, production of lymphocytotoxic antibodies and reaction to donor peripheral blood lymphocytes are monitored. Survival of a graft in this system for longer than nine days, without immunosuppressive therapy, indicates an increased level of tolerance.

In the 1E6 treated baboon, the transplanted allogeneic heart was still beating twenty-three days after the transplant. Thus, 1E6 dramatically improved tolerance for a cardiac allograft.

B. LFA3TIP Treatment

Using procedures substantially as described in Example 4A, the effect of LFA3TIP on cardiac allograft rejection is assessed. Purified LFA3TIP (described supra) is injected into one adult baboon at a dose of 3 mg/kg on day one for 2 consecutive days before the transplant. On the third day a cardiac heterotopic allograft transplant is performed with a heart from a young baboon. One dose of 3 mg/kg LFA3TIP is injected on the day of the transplant and then once a day for nine consecutive days.

The schedule of blood sample collection and analysis, and assessment of allograft rejection, is substantially as described in Example 4A.

Survival of the graft in the baboon that is treated with LFA3TIP is extended, compared to graft survival in untreated baboons, indicating increased graft tolerance due to LFA3TIP.

Deposits

Murine hybridoma cells and antibodies useful in the present invention are exemplified by cultures deposited under the Budapest Treaty with American Type Culture Collection, Rockville, Maryland, U.S.A., on March 5, 1991, and identified as:

Designation	ATCC Accession No.
1E6	HB 10393
HC-1B11	HB 10694
7A6	HB 10695
8B8	HB 10696

E. coli JA221 transformed with plasmid pSAB152 (encoding LFA3TIP) was deposited under the Budapest Treaty with American Type Culture Collection on October 1, 1991 and identified as:

Designation	ATCC Accession No.
pSAB152	68720

A bacteriophage carrying a plasmid encoding transmembrane LFA-3 was deposited under the Budapest Treaty with In Vitro International, Inc., Linthicum, Maryland, U.S.A., on May 28, 1987. This deposit was transferred to American Type Culture Collection on June 20, 1991 and identified as:

Designation	ATCC Accession No.
λHT16[λgt10/LFA-3]	75107

E. coli transformed with a plasmid encoding PI-linked LFA-3 was deposited under the Budapest Treaty with In Vitro International, Inc. on July 22, 1988. This deposit was transferred to American Type Culture Collection on June 20, 1991 and identified as:

Designation	ATCC Accession No.
p24	68788

Sequences

The following is a summary of the sequences set forth in the Sequence Listing:

SEQ ID NO:1 DNA sequence of transmembrane LFA-3
SEQ ID NO:2 Amino acid sequence of transmembrane LFA-3
SEQ ID NO:3 DNA sequence of PI-linked LFA-3
SEQ ID NO:4 Amino acid sequence of PI-linked LFA-3
SEQ ID NO:5 DNA sequence of CD2
SEQ ID NO:6 Amino acid sequence of CD2
SEQ ID NO:7 DNA sequence of LFA3TIP
SEQ ID NO:8 Amino acid sequence of LFA3TIP

While we have hereinbefore described a number of embodiments of this invention, it is apparent that our basic embodiments can be altered to provide other embodiments that utilize the processes of this invention. Therefore, it will be appreciated that the scope of this invention includes all alternative embodiments and variations which are defined in the foregoing specification and by the claims appended hereto; and the invention is not to be limited by the specific embodiments that have been presented herein by way of example.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: WALLNER, Barbara P.
BENJAMIN, Christopher D.

(ii) TITLE OF INVENTION: METHODS OF IMPROVING ALLOGRAFT OR XENOGRAFT TOLERANCE BY ADMINISTRATION OF LFA-3 OR CD2 BINDING PROTEINS

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: c\o FISH & NEAVE
(B) STREET: 875 Third Avenue
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: U.S.A.
(F) ZIP: 10022

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/772,705
(B) FILING DATE: 07-OCT-1991

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Haley Jr., James F.
(B) REGISTRATION NUMBER: 27,794
(C) REFERENCE/DOCKET NUMBER: B162CIP

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 715-0600

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 753 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..750

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..84

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 85..750

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..750
(D) OTHER INFORMATION: /note= "Human transmembrane LFA-3"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 646..714
(D) OTHER INFORMATION: /note= "Transmembrane domain"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATG GTT GCT GGG AGC GAC GCG GGG CGG GCC CTG GGG GTC CTC AGC GTG      48
Met Val Ala Gly Ser Asp Ala Gly Arg Ala Leu Gly Val Leu Ser Val
-28         -25                 -20                 -15

GTC TGC CTG CTG CAC TGC TTT GGT TTC ATC AGC TGT TTT TCC CAA CAA      96
Val Cys Leu Leu His Cys Phe Gly Phe Ile Ser Cys Phe Ser Gln Gln
        -10                 -5                   1

ATA TAT GGT GTT GTG TAT GGG AAT GTA ACT TTC CAT GTA CCA AGC AAT     144
Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His Val Pro Ser Asn
  5                  10                  15                  20

GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA AAG GAT AAA GTT GCA     192
Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys Asp Lys Val Ala
                 25                  30                  35

GAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA TCT TTT AAA AAT AGG     240
Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser Ser Phe Lys Asn Arg
            40                  45                  50

GTT TAT TTA GAC ACT GTG TCA GGT AGC CTC ACT ATC TAC AAC TTA ACA     288
Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr Ile Tyr Asn Leu Thr
        55                  60                  65

TCA TCA GAT GAA GAT GAG TAT GAA ATG GAA TCG CCA AAT ATT ACT GAT     336
Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro Asn Ile Thr Asp
    70                  75                  80

ACC ATG AAG TTC TTT CTT TAT GTG CTT GAG TCT CTT CCA TCT CCC ACA     384
Thr Met Lys Phe Phe Leu Tyr Val Leu Glu Ser Leu Pro Ser Pro Thr
 85                  90                  95                 100
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CTA ACT TGT GCA TTG ACT AAT GGA AGC ATT GAA GTC CAA TGC ATG ATA      432
Leu Thr Cys Ala Leu Thr Asn Gly Ser Ile Glu Val Gln Cys Met Ile
                105                 110                 115

CCA GAG CAT TAC AAC AGC CAT CGA GGA CTT ATA ATG TAC TCA TGG GAT      480
Pro Glu His Tyr Asn Ser His Arg Gly Leu Ile Met Tyr Ser Trp Asp
            120                 125                 130

TGT CCT ATG GAG CAA TGT AAA CGT AAC TCA ACC AGT ATA TAT TTT AAG      528
Cys Pro Met Glu Gln Cys Lys Arg Asn Ser Thr Ser Ile Tyr Phe Lys
        135                 140                 145

ATG GAA AAT GAT CTT CCA CAA AAA ATA CAG TGT ACT CTT AGC AAT CCA      576
Met Glu Asn Asp Leu Pro Gln Lys Ile Gln Cys Thr Leu Ser Asn Pro
    150                 155                 160

TTA TTT AAT ACA ACA TCA TCA ATC ATT TTG ACA ACC TGT ATC CCA AGC      624
Leu Phe Asn Thr Thr Ser Ser Ile Ile Leu Thr Thr Cys Ile Pro Ser
165                 170                 175                 180

AGC GGT CAT TCA AGA CAC AGA TAT GCA CTT ATA CCC ATA CCA TTA GCA      672
Ser Gly His Ser Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala
                185                 190                 195

GTA ATT ACA ACA TGT ATT GTG CTG TAT ATG AAT GGT ATT CTG AAA TGT      720
Val Ile Thr Thr Cys Ile Val Leu Tyr Met Asn Gly Ile Leu Lys Cys
            200                 205                 210

GAC AGA AAA CCA GAC AGA ACC AAC TCC AAT TGA                          753
Asp Arg Lys Pro Asp Arg Thr Asn Ser Asn
        215                 220
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 250 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Val Ala Gly Ser Asp Ala Gly Arg Ala Leu Gly Val Leu Ser Val
-28         -25                 -20                 -15

Val Cys Leu Leu His Cys Phe Gly Phe Ile Ser Cys Phe Ser Gln Gln
    -10                     -5                   1

Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His Val Pro Ser Asn
  5                  10                  15                  20

Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys Asp Lys Val Ala
                25                  30                  35
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Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser Ser Phe Lys Asn Arg
40 45 50

Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr Ile Tyr Asn Leu Thr
55 60 65

Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro Asn Ile Thr Asp
70 75 80

Thr Met Lys Phe Phe Leu Tyr Val Leu Glu Ser Leu Pro Ser Pro Thr
85 90 95 100

Leu Thr Cys Ala Leu Thr Asn Gly Ser Ile Glu Val Gln Cys Met Ile
105 110 115

Pro Glu His Tyr Asn Ser His Arg Gly Leu Ile Met Tyr Ser Trp Asp
120 125 130

Cys Pro Met Glu Gln Cys Lys Arg Asn Ser Thr Ser Ile Tyr Phe Lys
135 140 145

Met Glu Asn Asp Leu Pro Gln Lys Ile Gln Cys Thr Leu Ser Asn Pro
150 155 160

Leu Phe Asn Thr Thr Ser Ser Ile Ile Leu Thr Thr Cys Ile Pro Ser
165 170 175 180

Ser Gly His Ser Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala
185 190 195

Val Ile Thr Thr Cys Ile Val Leu Tyr Met Asn Gly Ile Leu Lys Cys
200 205 210

Asp Arg Lys Pro Asp Arg Thr Asn Ser Asn
215 220

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 723 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..720

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..84

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 85..720

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..720
(D) OTHER INFORMATION: /note= "Human PI-linked LFA-3"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 568..720
(D) OTHER INFORMATION: /note= "Signal sequence for PI-linkage"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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ATG GTT GCT GGG AGC GAC GCG GGG CGG GCC CTG GGG GTC CTC AGC GTG      48
Met Val Ala Gly Ser Asp Ala Gly Arg Ala Leu Gly Val Leu Ser Val
-28         -25                     -20                 -15

GTC TGC CTG CTG CAC TGC TTT GGT TTC ATC AGC TGT TTT TCC CAA CAA      96
Val Cys Leu Leu His Cys Phe Gly Phe Ile Ser Cys Phe Ser Gln Gln
        -10                  -5                   1

ATA TAT GGT GTT GTG TAT GGG AAT GTA ACT TTC CAT GTA CCA AGC AAT     144
Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His Val Pro Ser Asn
5                   10                  15                  20

GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA AAG GAT AAA GTT GCA     192
Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys Asp Lys Val Ala
                25                  30                  35

GAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA TCT TTT AAA AAT AGG     240
Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser Ser Phe Lys Asn Arg
            40                  45                  50

GTT TAT TTA GAC ACT GTG TCA GGT AGC CTC ACT ATC TAC AAC TTA ACA     288
Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr Ile Tyr Asn Leu Thr
        55                  60                  65

TCA TCA GAT GAA GAT GAG TAT GAA ATG GAA TCG CCA AAT ATT ACT GAT     336
Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro Asn Ile Thr Asp
    70                  75                  80

ACC ATG AAG TTC TTT CTT TAT GTG CTT GAG TCT CTT CCA TCT CCC ACA     384
Thr Met Lys Phe Phe Leu Tyr Val Leu Glu Ser Leu Pro Ser Pro Thr
85                  90                  95                 100

CTA ACT TGT GCA TTG ACT AAT GGA AGC ATT GAA GTC CAA TGC ATG ATA     432
Leu Thr Cys Ala Leu Thr Asn Gly Ser Ile Glu Val Gln Cys Met Ile
                105                 110                 115

CCA GAG CAT TAC AAC AGC CAT CGA GGA CTT ATA ATG TAC TCA TGG GAT     480
Pro Glu His Tyr Asn Ser His Arg Gly Leu Ile Met Tyr Ser Trp Asp
            120                 125                 130
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TGT CCT ATG GAG CAA TGT AAA CGT AAC TCA ACC AGT ATA TAT TTT AAG 528
Cys Pro Met Glu Gln Cys Lys Arg Asn Ser Thr Ser Ile Tyr Phe Lys
135 140 145

ATG GAA AAT GAT CTT CCA CAA AAA ATA CAG TGT ACT CTT AGC AAT CCA 576
Met Glu Asn Asp Leu Pro Gln Lys Ile Gln Cys Thr Leu Ser Asn Pro
150 155 160

TTA TTT AAT ACA ACA TCA TCA ATC ATT TTG ACA ACC TGT ATC CCA AGC 624
Leu Phe Asn Thr Thr Ser Ser Ile Ile Leu Thr Thr Cys Ile Pro Ser
165 170 175 180

AGC GGT CAT TCA AGA CAC AGA TAT GCA CTT ATA CCC ATA CCA TTA GCA 672
Ser Gly His Ser Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala
185 190 195

GTA ATT ACA ACA TGT ATT GTG CTG TAT ATG AAT GGT ATG TAT GCT TTT 720
Val Ile Thr Thr Cys Ile Val Leu Tyr Met Asn Gly Met Tyr Ala Phe
200 205 210

TAA 723

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 240 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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-28 -25 -20 -15

Val Cys Leu Leu His Cys Phe Gly Phe Ile Ser Cys Phe Ser Gln Gln
-10 -5 1

Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His Val Pro Ser Asn
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Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys Asp Lys Val Ala
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Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser Ser Phe Lys Asn Arg
40 45 50

Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr Ile Tyr Asn Leu Thr
55 60 65

Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro Asn Ile Thr Asp
70 75 80

Thr Met Lys Phe Phe Leu Tyr Val Leu Glu Ser Leu Pro Ser Pro Thr
85 90 95 100

Leu Thr Cys Ala Leu Thr Asn Gly Ser Ile Glu Val Gln Cys Met Ile
105 110 115

Pro Glu His Tyr Asn Ser His Arg Gly Leu Ile Met Tyr Ser Trp Asp
120 125 130

Cys Pro Met Glu Gln Cys Lys Arg Asn Ser Thr Ser Ile Tyr Phe Lys
135 140 145

Met Glu Asn Asp Leu Pro Gln Lys Ile Gln Cys Thr Leu Ser Asn Pro
150 155 160

Leu Phe Asn Thr Thr Ser Ser Ile Ile Leu Thr Thr Cys Ile Pro Ser
165 170 175 180

Ser Gly His Ser Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala
185 190 195

Val Ile Thr Thr Cys Ile Val Leu Tyr Met Asn Gly Met Tyr Ala Phe
200 205 210

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1056 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1053

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..72

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 73..1053

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..1053
(D) OTHER INFORMATION: /note= "Human CD2"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 628..702
(D) OTHER INFORMATION: /note= "Transmembrane domain"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
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GTT TCT TCC AAA GGT GCA GTC TCC AAA GAG ATT ACG AAT GCC TTG GAA      96
Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
            -5                  1               5

ACC TGG GGT GCC TTG GGT CAG GAC ATC AAC TTG GAC ATT CCT AGT TTT     144
Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe
    10                  15                  20

CAA ATG AGT GAT GAT ATT GAC GAT ATA AAA TGG GAA AAA ACT TCA GAC     192
Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
25                  30                  35                  40

AAG AAA AAG ATT GCA CAA TTC AGA AAA GAG AAA GAG ACT TTC AAG GAA     240
Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu
                45                  50                  55

AAA GAT ACA TAT AAG CTA TTT AAA AAT GGA ACT CTG AAA ATT AAG CAT     288
Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His
            60                  65                  70

CTG AAG ACC GAT GAT CAG GAT ATC TAC AAG GTA TCA ATA TAT GAT ACA     336
Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr
        75                  80                  85

AAA GGA AAA AAT GTG TTG GAA AAA ATA TTT GAT TTG AAG ATT CAA GAG     384
Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu
    90                  95                  100

AGG GTC TCA AAA CCA AAG ATC TCC TGG ACT TGT ATC AAC ACA ACC CTG     432
Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu
105                 110                 115                 120

ACC TGT GAG GTA ATG AAT GGA ACT GAC CCC GAA TTA AAC CTG TAT CAA     480
Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln
                125                 130                 135

GAT GGG AAA CAT CTA AAA CTT TCT CAG AGG GTC ATC ACA CAC AAG TGG     528
Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp
            140                 145                 150

ACC ACC AGC CTG AGT GCA AAA TTC AAG TGC ACA GCA GGG AAC AAA GTC     576
Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val
        155                 160                 165

AGC AAG GAA TCC AGT GTC GAG CCT GTC AGC TGT CCA GAG AAA GGT CTG     624
Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro Glu Lys Gly Leu
    170                 175                 180
```

GAC ATC TAT CTC ATC ATT GGC ATA TGT GGA GGA GGC AGC CTC TTG ATG 672
Asp Ile Tyr Leu Ile Ile Gly Ile Cys Gly Gly Gly Ser Leu Leu Met
185 190 195 200

GTC TTT GTG GCA CTG CTC GTT TTC TAT ATC ACC AAA AGG AAA AAA CAG 720
Val Phe Val Ala Leu Leu Val Phe Tyr Ile Thr Lys Arg Lys Lys Gln
205 210 215

AGG AGT CGG AGA AAT GAT GAG GAG CTG GAG ACA AGA GCC CAC AGA GTA 768
Arg Ser Arg Arg Asn Asp Glu Glu Leu Glu Thr Arg Ala His Arg Val
220 225 230

GCT ACT GAA GAA AGG GGC CGG AAG CCC CAC CAA ATT CCA GCT TCA ACC 816
Ala Thr Glu Glu Arg Gly Arg Lys Pro His Gln Ile Pro Ala Ser Thr
235 240 245

CCT CAG AAT CCA GCA ACT TCC CAA CAT CCT CCT CCA CCA CCT GGT CAT 864
Pro Gln Asn Pro Ala Thr Ser Gln His Pro Pro Pro Pro Pro Gly His
250 255 260

CGT TCC CAG GCA CCT AGT CAT CGT CCC CCG CCT CCT GGA CAC CGT GTT 912
Arg Ser Gln Ala Pro Ser His Arg Pro Pro Pro Pro Gly His Arg Val
265 270 275 280

CAG CAC CAG CCT CAG AAG AGG CCT CCT GCT CCG TCG GGC ACA CAA GTT 960
Gln His Gln Pro Gln Lys Arg Pro Pro Ala Pro Ser Gly Thr Gln Val
285 290 295

CAC CAG CAG AAA GGC CCG CCC CTC CCC AGA CCT CGA GTT CAG CCA AAA 1008
His Gln Gln Lys Gly Pro Pro Leu Pro Arg Pro Arg Val Gln Pro Lys
300 305 310

CCT CCC CAT GGG GCA GCA GAA AAC TCA TTG TCC CCT TCC TCT AAT 1053
Pro Pro His Gly Ala Ala Glu Asn Ser Leu Ser Pro Ser Ser Asn
315 320 325

TAA 1056

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 351 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
-24 -20 -15 -10

Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
-5 1 5

```
Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe
    10                  15                  20

Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
25                  30                  35                  40

Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu
                45                  50                  55

Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His
            60                  65                  70

Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr
        75                  80                  85

Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu
    90                  95                  100

Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu
105                 110                 115                 120

Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln
                125                 130                 135

Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp
            140                 145                 150

Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val
        155                 160                 165

Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro Glu Lys Gly Leu
    170                 175                 180

Asp Ile Tyr Leu Ile Ile Gly Ile Cys Gly Gly Gly Ser Leu Leu Met
185                 190                 195                 200

Val Phe Val Ala Leu Leu Val Phe Tyr Ile Thr Lys Arg Lys Lys Gln
                205                 210                 215

Arg Ser Arg Arg Asn Asp Glu Glu Leu Glu Thr Arg Ala His Arg Val
            220                 225                 230

Ala Thr Glu Glu Arg Gly Arg Lys Pro His Gln Ile Pro Ala Ser Thr
        235                 240                 245

Pro Gln Asn Pro Ala Thr Ser Gln His Pro Pro Pro Pro Pro Gly His
    250                 255                 260

Arg Ser Gln Ala Pro Ser His Arg Pro Pro Pro Pro Gly His Arg Val
265                 270                 275                 280

Gln His Gln Pro Gln Lys Arg Pro Pro Ala Pro Ser Gly Thr Gln Val
                285                 290                 295
```

His Gln Gln Lys Gly Pro Pro Leu Pro Arg Pro Arg Val Gln Pro Lys
300 305 310

Pro Pro His Gly Ala Ala Glu Asn Ser Leu Ser Pro Ser Ser Asn
315 320 325

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1050 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1041

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..84

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 85..1041

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 85..1041
(D) OTHER INFORMATION: /note= "LFA3TIP"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 360..361
(D) OTHER INFORMATION: /note= "LFA-3/IgG fusion point"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATG GTT GCT GGG AGC GAC GCG GGG CGG GCC CTG GGG GTC CTC AGC GTG 48
Met Val Ala Gly Ser Asp Ala Gly Arg Ala Leu Gly Val Leu Ser Val
-28 -25 -20 -15

GTC TGC CTG CTG CAC TGC TTT GGT TTC ATC AGC TGT TTT TCC CAA CAA 96
Val Cys Leu Leu His Cys Phe Gly Phe Ile Ser Cys Phe Ser Gln Gln
-10 -5 1

ATA TAT GGT GTT GTG TAT GGG AAT GTA ACT TTC CAT GTA CCA AGC AAT 144
Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His Val Pro Ser Asn
5 10 15 20

GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA AAG GAT AAA GTT GCA 192
Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys Asp Lys Val Ala
25 30 35

GAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA TCT TTT AAA AAT AGG 240
Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser Ser Phe Lys Asn Arg
40 45 50

GTT TAT TTA GAC ACT GTG TCA GGT AGC CTC ACT ATC TAC AAC TTA ACA 288
Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr Ile Tyr Asn Leu Thr
55 60 65

TCA TCA GAT GAA GAT GAG TAT GAA ATG GAA TCG CCA AAT ATT ACT GAT 336
Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro Asn Ile Thr Asp
70 75 80

ACC ATG AAG TTC TTT CTT TAT GTC GAC AAA ACT CAC ACA TGC CCA CCG 384
Thr Met Lys Phe Phe Leu Tyr Val Asp Lys Thr His Thr Cys Pro Pro
85 90 95 100

TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC 432
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
105 110 115

CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA 480
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
120 125 130

TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC 528
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
135 140 145

TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG 576
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
150 155 160

GAG GAG CAG TAC AAC AGC ACG TAC CGG GTG GTC AGC GTC CTC ACC GTC 624
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
165 170 175 180

CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC 672
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
185 190 195

AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA 720
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
200 205 210

GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT 768
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
215 220 225

GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC 816
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
230 235 240

TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG 864
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
245 250 255 260

AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC 912
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
265 270 275

TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG 960
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
280 285 290

AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC 1008
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
295 300 305

ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGAGTGCGG 1050
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
310 315

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 347 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Val Ala Gly Ser Asp Ala Gly Arg Ala Leu Gly Val Leu Ser Val
-28 -25 -20 -15

Val Cys Leu Leu His Cys Phe Gly Phe Ile Ser Cys Phe Ser Gln Gln
-10 -5 1

Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His Val Pro Ser Asn
5 10 15 20

Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys Asp Lys Val Ala
25 30 35

Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser Ser Phe Lys Asn Arg
40 45 50

Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr Ile Tyr Asn Leu Thr
55 60 65

Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro Asn Ile Thr Asp
70 75 80

Thr Met Lys Phe Phe Leu Tyr Val Asp Lys Thr His Thr Cys Pro Pro
85 90 95 100

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
105 110 115

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
120 125 130

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
135 140 145

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
150 155 160

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
165 170 175 180

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
185 190 195

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
200 205 210

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
215 220 225

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
230 235 240

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
245 250 255 260

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
265 270 275

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
280 285 290

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
295 300 305

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
310 315

We claim:

1. A method for improving tolerance of transplanted allograft tissue or xenograft tissue comprising the steps of administering to a mammal, including a human, the graft tissue and an LFA-3 or CD2 binding protein.

2. The method according to claim 1, wherein the binding protein inhibits T cell activation.

3. The method according to claim 1, wherein an LFA-3 binding protein is administered.

4. The method according to claim 3, wherein the LFA-3 binding protein is a soluble CD2 polypeptide.

5. The method according to claim 3, wherein the LFA-3 binding protein is a monoclonal anti-LFA-3 antibody.

6. The method according to claim 5, wherein the monoclonal anti-LFA-3 antibody is produced by a hybridoma selected from hybridomas having accession numbers ATCC HB 10693 (1E6), ATCC HB 10694 (HC-1B11), ATCC HB 10695 (7A6), ATCC HB 10696 (8B8) or is the monoclonal antibody TS2/9.

7. The method according to claim 6, wherein the monoclonal anti-LFA-3 antibody is produced by the hybridoma having accession number ATCC HB 10693 (1E6).

8. The method according to claim 1, wherein a CD2 binding protein is administered.

9. The method according to claim 8, wherein the CD2 binding protein is a monoclonal anti-CD2 antibody.

10. The method according to claim 8, wherein the CD2 binding protein is a soluble LFA-3 polypeptide.

11. The method according to claim 10, wherein the soluble LFA-3 polypeptide is selected from the group of polypeptides consisting of AA_1-AA_{92} of SEQ ID NO:2, AA_1-AA_{80} of SEQ ID NO:2, AA_{50}-AA_{65} of SEQ ID NO:2, and AA_{20}-AA_{80} of SEQ ID NO:2.

12. The method according to claim 11, wherein the LFA-3 polypeptide is AA_1-AA_{92} of SEQ ID NO:2.

13. The method according to claim 1, wherein the binding protein is a humanized recombinant antibody.

14. The method according to claim 1, wherein the binding protein is a chimeric recombinant antibody.

15. The method according to claim 5 or 9, wherein the binding protein is selected from Fab fragments, Fab' fragments, $F(ab')_2$ fragments, F(v) fragments and intact immunoglobulin heavy chains of the anti-LFA-3 or anti-CD2 monoclonal antibody.

16. The method according to claim 15, wherein the binding protein is selected from monomers and dimers of full length immunoglobulin heavy chains.

17. The method according to claim 1, wherein the graft tissue is a xenograft.

18. The method according to claim 17, wherein the graft tissue is a cardiac or renal xenograft.

19. The method according to claim 1, wherein the graft tissue is an allograft.

20. The method according to claim 19, wherein the graft tissue is a cardiac or renal allograft.

21. The method according to claim 1, wherein the mammal is a human.

22. The method according to claim 1, wherein the graft tissue is perfused with an effective amount of the binding protein before implantation into the mammal.

23. The method according to claim 1, wherein the binding protein is administered at a dose between about 0.01 and about 10 mg binding protein/kg body weight.

24. The method according to claim 3, wherein the LFA-3 binding protein is administered at a dose between about 0.1 and about 5 mg binding protein/kg body weight.

25. The method according to claim 24, wherein LFA-3 binding protein is administered at a dose

between about 0.1 and about 2 mg binding protein/kg body weight.

26. The method according to claim 8, wherein the CD2 binding protein is administered at a dose between about 0.01 and about 2 mg binding protein/kg body weight.

27. The method according to claim 26, wherein the CD2 binding protein is administered at a dose between about 0.01 and about 1 mg binding protein/kg body weight.

28. The method according to claim 19, wherein the binding protein is administered once per day for two consecutive days before the transplant and once per day for one to ten consecutive days after the transplant.

29. The method according to claim 28, wherein the binding protein is administered once per day for two consecutive days before the transplant and once per day for two consecutive days after the transplant.

30. The method according to claim 17, wherein before the transplant the binding protein is administered contemporaneously with tissue from the xenograft source.

31. The method according to claim 30, wherein the contemporaneous administration is followed by administration before the transplant of the binding protein.

32. The method according to claim 17, wherein the binding protein is administered before the transplant once per day for two consecutive days, then contemporaneously with tissue from the xenograft source once per day for one day and then once per day for one to ten consecutive days.

33. The method according to claim 32, wherein the binding protein is administered before the transplant once per day for two consecutive days, then contemporaneously with tissue from the xenograft source once per day for one day and then once per day for five to ten consecutive days.

34. The method according to claim 31, wherein the contemporaneous administration of the binding protein and the tissue from the xenograft source is simultaneous.

35. The method according to claim 30 or 32, wherein the tissue from the xenograft source is blood.

36. The method according to claim 1, wherein the binding protein is administered intravenously, intramuscularly, subcutaneously, intra-articularly, intrathecally, periostally, orally, topically or by inhalation.

37. The method according to claim 36, wherein the binding protein is administered intravenously or intramuscularly.

38. The method according to claim 1, wherein the binding protein is administered with an effective amount of an immunosuppressive agent.

39. The method according to claim 38, wherein the immunosuppressive agent is cyclosporine.

40. The method according to claim 38, wherein the immunosuppressive agent is prednisone.

41. The method according to claim 38, wherein the immunosuppressant agent is prednisone and cyclosporine.

42. The method according to claim 1, wherein the binding protein is linked to one or more members selected from the group consisting of LFA-3 binding proteins, CD2 binding proteins and pharmaceutical agents.

43. The method according to claim 42, wherein the binding protein is a soluble LFA-3 polypeptide linked to a human immunoglobulin heavy chain hinge region and constant region, or portions thereof.

44. The method according to claim 43, wherein the soluble LFA-3 polypeptide is selected from the group consisting of AA_1-AA_{92} of SEQ ID NO:2, AA_1-AA_{80} of SEQ ID NO:2, AA_{50}-AA_{65} of SEQ ID NO:2, and AA_{20}-AA_{80} of SEQ ID NO:2.

45. The method according to claim 44, wherein the soluble LFA-3 polypeptide is AA_1-AA_{92} of SEQ ID NO:2.

46. The method according to claim 45, wherein the binding protein consists of AA_1-AA_{319} of SEQ ID NO:8.

FIG. 1

T CELL DEPENDENT B CELL ACTIVATION ASSAY

BABOON B (1E6)

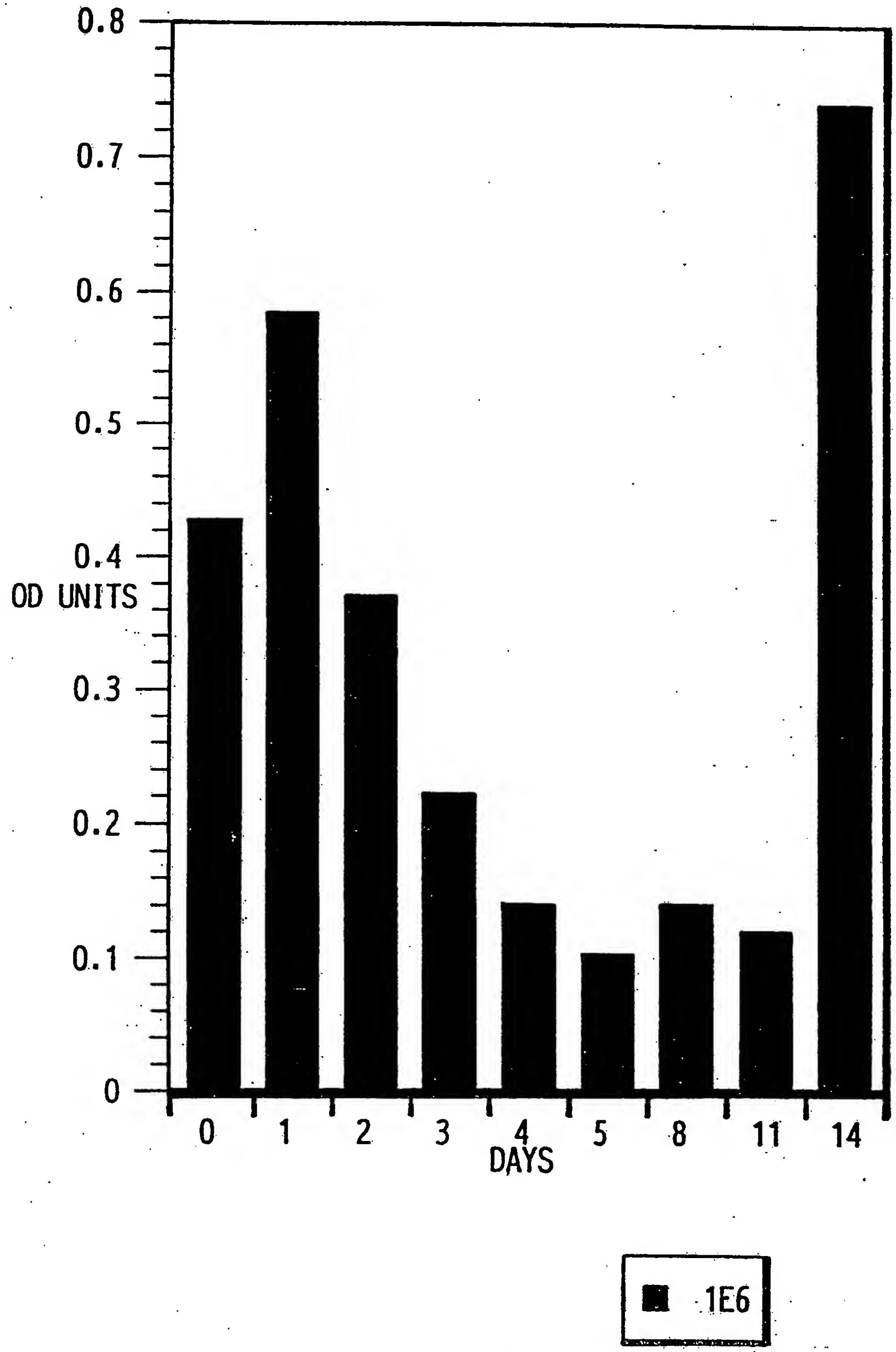

FIG. 2

T CELL DEPENDENT B CELL ACTIVATION ASSAY

BABOONS C (MOPC21) AND A (1E6)